STAT

LYSERGIC ACID AS A BASIS IN THE COLORIMETRIC DETERMINATION OF ERGOT ALKALOIDS

Acta Chim. Hung.,
Vol. 1, No. 1. 1952,
Budapest, pages 15-73

Pal Tuzson, Jolan Tuzson,
Istvan Bayer, Chemistry Laboratory
of the Richter Company and
Chemistry Department of the
State Hygiene Institute, Budapest

Received: 24 July 1951

Because of the outstanding role of ergot alkaloids in medicine, and because of their great value, increasing demands are being made on production control of these pharmaceuticals. This task is however complicated by the inconvenience due to the exposure of analytical grade preparations to standard tests, plus the fact that they do not remain stable for very long, even when hermetically sealed and in darkness. The authors have therefore tried to determine experimentally whether a single, relatively stable standard is usable for the determination of all the ergot alkaloids.

Photometric determination is based, as is generally known, on the Van Urk color reaction (1). The blue color results from the effect of p-dimethylamidobenzaldehyde in sulfuric acid solution on β-substituted indole derivatives. In the given case the lysergic acid which forms part in the building up of all ergot alkaloids is the cause of this reaction. Since the mechanism of this reaction is unknown, it was questionable whether perhaps other groups and parts of the molecule influence the color either in a qualitative or a quantitative sense. Smith and Timmis (2) mention that the intensity of the color, in the case of the ergine produced by isomerization (isolysergic acid amide), and of ergotrinine, is in indirect proportion to the molar weight. Since ergotrinine is the amide of isolysergic acid formed with a relatively small

accompanying extinctions are drawn next to the lysergic acid line (Figure 2), then it can be seen that the 2 lines lie within the range of experimental error. The deviation amounts to an average of 3.7%. In the case of ergoclavine the extinctions belonging to the converted values (designated by +) fall to the lysergic acid line.

The results therefore prove with certainty that the color reaction is dependent only on the lysergic acid part of the molecule and is not influenced by the shorter or longer side chain either in a qualitative or quantitative sense.

This proves also -- according to our own experiments -- that ergotinine, on the basis of its construction and its rotatibility, can be looked upon as pure ergocristinine ($C_{35}H_{39}O_5N_5$; molar weight = 609.35) (see experimental part). The experimentally derived molar weight, calculated from lines 1 and 4 of Figure 1, shows an average of 623.5, with a deviation of 2.3%. The molar weight of ergocryptinine and ergocorninine (the components of so-called pseudo-ergotinine) are significantly smaller (575.37 and 561.35 respectively).

But the ergotoxine preparations already consist of a mixture of 3 different alkaloids in varying amounts;

Ergotoxine phosphate:

Ergocristine phosphate $C_{35}H_{39}O_5N_5 \cdot H_3PO_4 \cdot H_2O$ Molar weight 725.43

Ergokryptine phosphate $C_{32}H_{41}O_5N_5 \cdot H_3PO_4 \cdot H_2O$ Molar weight 691.45

Ergocorninine phosphate $C_{31}H_{39}O_5N_5 \cdot H_3PO_4 \cdot H_2O$ Molar weight 677.43

Dihydro-ergotoxine phosphate:

Dihydro-ergocristine phosphate $C_{35}H_{41}O_5N_5 \cdot H_3PO_4 \cdot H_2O$ Molar weight 727.45

Dihydro-ergocryptinine phosphate $C_{32}H_{43}O_5N_5 \cdot H_3PO_4 \cdot H_2O$ Molar weight 693.46

Dihydro-ergocornine phosphate $C_{31}H_{41}O_5N_5 \cdot H_3PO_4 \cdot H_2O$ Molar weight 679.45

accompanying extinctions are drawn next to the lysergic acid line (Figure 2), then it can be seen that the 2 lines lie within the range of experimental error. The deviation amounts to an average of 3.7%. In the case of ergoclavine the extinctions belonging to the converted values (designated by +) fall to the lysergic acid line.

The results therefore prove with certainty that the color reaction is dependent only on the lysergic acid part of the molecule and is not influenced by the shorter or longer side chain either in a qualitative or quantitative sense.

This proves also -- according to our own experiments -- that ergotinine, on the basis of its construction and its rotatibility, can be looked upon as pure ergocristinine ($C_{35}H_{39}O_5N_5$; molar weight = 609.35) (see experimental part). The experimentally derived molar weight, calculated from lines 1 and 4 of Figure 1, shows an average of 623.5, with a deviation of 2.3%. The molar weight of ergocryptinine and ergocorninine (the components of so-called pseudo-ergotinine) are significantly smaller (575.37 and 561.35 respectively).

But the ergotoxine preparations already consist of a mixture of 3 different alkaloids in varying amounts;

Ergotoxine phosphate:

Ergocristine phosphate $C_{35}H_{39}O_5N_5 \cdot H_3PO_4 \cdot H_2O$ Molar weight 725.43

Ergokryptine phosphate $C_{32}H_{41}O_5N_5 \cdot H_3PO_4 \cdot H_2O$ Molar weight 691.45

Ergocorninine phosphate $C_{31}H_{39}O_5N_5 \cdot H_3PO_4 \cdot H_2O$ Molar weight 677.43

Dihydro-ergotoxine phosphate:

Dihydro-ergocristine phosphate $C_{35}H_{41}O_5N_5 \cdot H_3PO_4 \cdot H_2O$ Molar weight 727.45

Dihydro-ergocryptinine phosphate $C_{32}H_{43}O_5N_5 \cdot H_3PO_4 \cdot H_2O$ Molar weight 693.46

Dihydro-ergocornine phosphate $C_{31}H_{41}O_5N_5 \cdot H_3PO_4 \cdot H_2O$ Molar weight 679.45

In the ergotoxine samples analyzed by Stoll (3), ergocristine and ergocornine are alternately dominant, and the ergocryptine, having an average molar weight, appears only in a relatively minor quantity.

The molar weight of the ergotoxine phosphate investigated by the authors, calculated from lines 1 and 5 of Figure 1, shows an average of 681.9. The heavily drawn line in Figure 3 corresponding to this value falls between the ideal extinction curves, calculated on the basis of lysergic acid content of the C_{35} and C_{31} components, and actually quite close to the ergocornine phosphate with its low molar weight. Since the deviation of the 2 extreme molar weights is rather large (6.62%), and in fact greater than the measuring error of colorimetry, it can be asserted that the ergotoxine preparation under investigation is rich in ergocornine.

The result of the experiment with respect to dihydro-ergotoxine phosphate is of principal importance. Here again the question is posed whether the saturation of double bonding in the hydroaromatic part of the lysergic acid does not perhaps influence the color reaction. (The water-soluble salts of dihydro-ergotoxine are widely known, strongly hypotensive pharmaceuticals. Hydergine is its methane sulfonate.)

From Figure 4 it can be seen that the extinction line of the dihydro-ergotoxine phosphate investigated by the authors, like the previous case, falls between the curves calculated for the C_{35} and C_{31} components, and therefore the hydration of the lysergic acid part has no influence on the formation of the color.

According to the results above therefore lysergic acid, as

well as any homogeneous, chemically pure alkaloid (or its salt), is usable as a standard for the colorimetric determination of ergot alkaloids. Any alkaloid can be evaluated by simple calculation from the graph which has been plotted for the purpose. It must however be emphasised that one can only use an average molar weight for calculation in the analysis of ergotoxine, since it is a mixture. In the cases which have been studied this comes very close to the arithmetical mean of the molar weights of ergocristine and ergocornine. The deviation in the case of ergotoxine phosphate amounts to 2.8% in the case of dihydro-ergotoxine phosphate 1.4%. Taking this average molar weight into account, one can evaluate the ergotoxine preparations with the help of the graph without appreciable error, since this graph can be plotted with any homogeneous alkaloid.

Experimental Part

For the absolute colorimetric measurements the process worked out by Schulek and Vastagh (5) was used with the aid of the Zeiss-Pulfrich photometer. Water as well as one % tartaric acid was used as a solvent. It was established by means of special series of experiments that the reaction is the same in both cases, assuming that the purity of the tartaric acid corresponds to the requirements of the pharmacopeia. Impurities cut down on the extinction. It is especially important that the nitrogen superoxide used for oxydation actually be 30%, since the yields are smaller with smaller concentration. The same pipettes were always used for diluting in order to avoid dropper error. Molnar and Uskert (6) used manganese sulfate and ferric (III)-ammonium sulfate as a catalyst in order to insure the smooth progress of the color reaction. But the authors found no difference in parallel experiments within the error limits with or without catalysts.

I. Lysergic Acid

The process was carried out according to Jacobs and Craig (7) by reduction with potassium hydroxide. Crystalline ergotinine was not used as the initial substance, but rather an industrially produced 80% ergot alkaloid mixture. The decomposition product was processed much more simply than as described in the cited prescription, by making use of the high adsorption potential of lysergic acid on activated charcoal. The resinous products were removed by filtration of the dark water solution which was obtained with the potassium hydroxide, and the solution was treated repeatedly with activated charcoal (Ipagite) until the Keller-Kiliani reaction was no longer present in the filtrated solution. The charcoal was thoroughly washed with water and exhaustively elutriated boiling absolute alcohol containing 10% ammonia. The ammoniated solution was concentrated, diluted with a little alcohol, and very weakly acidified with acetic acid. After the inoculation, the precipitation of lysergic acid began immediately. For the purpose of purification the acid was dissolved in potassium hydroxide and precipitated with sulphuric acid. After crystallization with hot water the analytical grade lysergic acid precipitated in well-formed hexagonal crystals. Melting point: 238° (decomposing). The water of crystallization was removed at 140° and 2 mm pressure.

$C_{16}H_{16}O_2N_2 \cdot H_2O$ Calculated: 6.29% H_2O

Actual: 6.50% H_2O

N-amount 1. 4.040 mg: 0.367 ml N_2 (751 mm, 22° C)

2. 5.087 mg: 0.466 ml N_2 (751 mm, 22° C)

C-H-amount 1. 3.467 mg: 9.132 mg CO_2, 1.937 mg H_2O

2. 3.899 mg: 10.153 mg CO_2, 2.196 mg H_2O

$C_{16}H_{16}O_2N_2$ Calculated: 7.69% C, 6.00% H, 10.45% N

Actual: 1. 71. % C, 6.2?% H, 10.20% N

?. 71.97% C, 6.30% H, 10.30% N

For colorimetry, 10.66 and 11.22 mg, respectively, of lysergic acid (I and II) were dissolved in 1% tartaric acid solution and filled to 500 ml. Table 1 contains the quantities in γ taken from these solutions, and the average values of the observed extinctions. The extinctions of solution I were determined 3 times: directly after the release (a), on the next day (b), and after 7 days (c). The solution remained in the flask with a glass stopper, in the dark, without fungus formation and -- as shown by the numerical table -- unchanged.

TABLE I

Lysergic acid Solution I γ	Extinction (average value) a	b	c	Lysergic acid Solution II γ	Extinction (average value)
10.66	0.0?9	--	--	22.44	0.19?
21.32	0.172	0.172	0.177	44.??	0.4??
31.98	0.277	--	--	67.32	0.?39
37.31	0.327	--	--		
42.64	0.393	0.402	0.400		
53.30	0.479	0.479	--		
5?.61	0.534	--	--		
63.96	0.600	0.610	0.610		
?6.28	0.?00	--	--		

II. Ergometrine Maleate

One g of commercially produced, pale cream-colored preparation was recrystallized from 100 ml of hot alcohol, which yielded long colorless rods of the substance.

Amount 1. 23. [illegible] mm, 24° C;

2. 27.6 mg [illegible] ml N_2 (746.7 mm, 24° C)

$C_{19}H_{23}O_2N_3 \cdot C_4H_4O_4$ Calculated: 9.52% N

Actual: 1. 9.35% N

2. 9.51% N

For colorimetry 15.4 mg were dissolved in water and the solution increased to 500 ml.

Ergometrine maleate (γ)	Extinction (Average value)
30.8	0.160
61.6	0.355
92.4	0.545
123.2	0.745

III. Ergoclavine

Ergoclavine was obtained from a raw ergot base mixture according to the Hungarian patent of E. Merck Company No 113,031. The tartaric acid solution of raw alkaloids was treated with 30% caustic soda up to alkali reaction, then the ergotinine was drawn off, and then, after acidification with lactic acid, the ergotoxine was drawn off. Then the solution, treated with sodium carbonate until alkaline reaction, was finally precipitated with chloroform and shaken out. The chloroform extraction was dried and concentrated, and for the purpose of purification the solution was poured over a short pile of von Brockmann standardized aluminum oxide. The solution was now straw-yellow. After drying, the substance was twice recrystallised from benzene. The ergoclavine thus obtained forms white, tiny, hexagonal tablets. Nor did the preparation become discolored after long exposure to the air, a rarity with the ergot alkaloids. It melts, with decomposition, at 175°.

Rotation in chloroform: $[\alpha]_D^{20°} = +109.2°$ (one % solution)

N-amount 1. 19.6 mg: 2.32 ml N_2 (753.2 mm, 30° C)

2. 19.4 mg: 2.20 ml N_2 (753.2 mm, 28° C)

$C_{30}H_{37}O_5N_5$ Calculated: 12.76% N

Actual: 1. 12.72% N

2. 12.58% N

For colorimetry 20.8 mg (I) and 28.2 mg (II) of ergoclavine were dissolved in 500 ml of one % tartaric acid solution.

Ergoclavine (γ) Solution (I)	Extinction (Average value)	Ergoclavine (γ) Solution (II)	Extinction (Average value)
41.6	0.160	56.4	0.245
62.4	0.259	84.6	0.374
83.2	0.379	112.8	0.517
104.0	0.460	141.0	0.624
124.8	0.573	169.2	0.768
166.4	0.746	221.6	0.510

IV. Ergotinine

The liquor of the ergotoxine phosphate extract (see below) was treated with $NaHCO_3$ up to alkaline reaction and drawn off with ether. The distillation residue, purified on an Al_2O_3 pile by chromatographic adsorption, was crystallized from aqueous alcohol. The ergotinine thus obtained formed large, pale green tablets. The following procedure was found to be very advantageous for further purification. The substance was dissolved in heated benzene, and the filtrated, still warm solution was diluted with light benzene until incipient cloudiness. From the cooled solution ergotinine precipitated in nicely formed prisms. The preparation is colorless, melts at 220-225° with decomposition. Rotation in

chloroform: $[a]_D^{20}$ [sic] = + 361° (0.4% solution). For the ergotinine crystallized from alcohol, Smith and Timmis (8) indicate a rotation of + 365°, and distinguish this from the pseudo ψ-ergotinine which they obtained from the more easily soluble fractions, and the rotation of which in chloroform solution is appreciably higher (+409°) under similar conditions. This deviation, according to Stoll (9), is derived from the fact that the ergotinine with smaller rotatibility is essentially pure ergocristinine, and the ψ ergotinine is an ergocorninine contaminated with ergocryptinine.

The dissolving of the ergotinine involved difficulties and was only achieved by dissolving the sample of 15.3 mg first in one ml of 10% alcoholic ethane-sulfonic acid and then diluting with one % tartaric acid to 250 ml, accompanied by careful agitation.

Ergotinine (γ)	Extinction (Average value)
61.2	0.224
91.8	0.355
122.4	0.473
153.0	0.600
183.6	0.730

V. Ergotoxine Phosphate

The raw ergot base mixture was digested with a triple quantity of methanol (10) and after dilution with acetone the ergotoxine phosphate was precipitated from the filtrate with alcoholic phosphoric acid (11). Crystallisation from alcohol is suitable for purification. Large hexagonal tablets and piles are thus obtained. Melting point 184-186° with decomposition.

Then 26.1 mg were dissolved in 2 ml of 2.5% alcoholic ethane-sulfonic

acid and the solution was diluted to 500 ml with one % tartaric acid solution.

Ergotoxine phosphate (γ)	Extinction (average value)
52.2	0.167
104.4	0.319
156.5	0.575
208.8	0.776

VI. Dihydro-ergotoxine phosphate

Pure ergotoxine phosphate was dissolved in a lukewarm mixture of a 20-unit quantity of dioxane and a 5-unit quantity of water. After addition of a half quantity of freshly prepared Pd sponge, the compound was hydrated for 6 hours in a bomb flask under 50 atm pressure at 40-45° C. The solution was filtrated, dried, and the residue taken up in water with weak acetic acid and treated with ammonia until alkaline reaction, then transferred to chloroform. The dried solution was poured over an Al_2O_3 pile and the colored product of decomposition was removed. From the acetone solution of the dry residue, dihydro-ergotoxine phosphate in nice crystals was obtained with alcoholic phosphoric acid. Melting point with decomposition is 198-199°, thus considerably higher than the melting point of the ergotoxine phosphate.

Saturation of the hydrogenable double bond of lysergic acid is most surely recognizable by the change of physiological action, since no demonstrable physiochemical difference exists between initial substance and hydrated product.

Biological comparison of ergotoxine phosphate (EPh) and dihydro-ergotoxine phosphate (DEPh):

Toxicity (LD_{50}): EPh 25mg/kg white mouse i. p. 72h

DEPh 100mg/kg white mouse i. p. 72h

On an isolated rabbit uterus:

EPh 0.0225 mg/kg dosage: sympathicolytic effect

DEPh 0.0150 mg/kg dosage: sympathicolytic effect

Blood pressure of the Koetst [sic]:

EPh 0.5 mg/kg dosage: strong blood-pressure increasing and sympathicolytic effect

DEPh 0.3 mg/kg dosage: strong blood-pressure decreasing and sympathicolytic effect.

For the colorimetric measurements 2.5 mg of the substance were dissolved in 500 ml of 1% tartaric acid solution.

Dihydro-ergotoxine phosphate (γ)	Extinction (average value)
43.0	0.131
64.5	0.209
86.0	0.290
107.5	0.360
129.0	0.450
172.0	0.600

The microelementary analyses were carried out in the Institute of Organic Chemistry, Eotvos Lorand University, Budapest, and partly by doctor Alajos Valy (Richter); the biological experiments were conducted by doctor Gabriela Zemplen (Richter).

Summary

The extinctions of solutions of lysergic acid with varying concentration, as well as ergometrine maleate, ergoclavine, and

ergotinine (ergocristinine) were plotted and determined with a Pulfrich photometer so that the blue color reaction formed with p-dimethyl amidobensaldehyde is produced only by the lysergic acid residue. Thus the colorimetric determination of ergo alkaloids is feasible with the help of this reaction, and as a standard, lysergic acid or any chemically homogeneous alkaloid (ergometrine maleate, ergoclavine, etc) can be used. The intensity of the blue color is inversely proportional to the molecular size. The depth of color is not affected by saturation of the hydrogenous double bond of lysergic acid.

BIBLIOGRAPHY

1. Van Urk, Pharm. Weekbl., 66, 473, 1929; Schmidt, M. S., US Public Health Reports, 1466, 1930
2. Smith S., Timmis, G. M., J. Chem Soc., 1166, 1936
3. Stoll, A., Hofmann, A., Helv. Chim. A., 26, 1570, 1943
4. Molnar, I., Uskert, A., Magyar Kemiai Folyoirat, 56, 230, 1950
5. Schulek, E., Vastagh, G., Magyar Gyogyszereszstud. Ert., 15, 322, 1939; Dansk Tidskrift for Farmaci, 13, 5, 1939
6. See item 4
7. Jacobs, W. A., Craig, L. C., J. Biol. Chem., 104, 547, 1934
8. Smith, S., Timmis, G. M., J. Chem Soc., 1888, 1931
9. See item 3
10. Smith, S., Timmis, G. M., J. Chem Soc., 1390, 1930
11. Barger, G., Carr, F. H., J. Chem Soc., 337, 1907
12. Jacobs, W. A., Craig, L. C., J. Biol. Chem., 115, 227, 1936; Stoll, A., Hofmann, A., Helv. Chim. A., 26, 2070, 1943

FIGURES

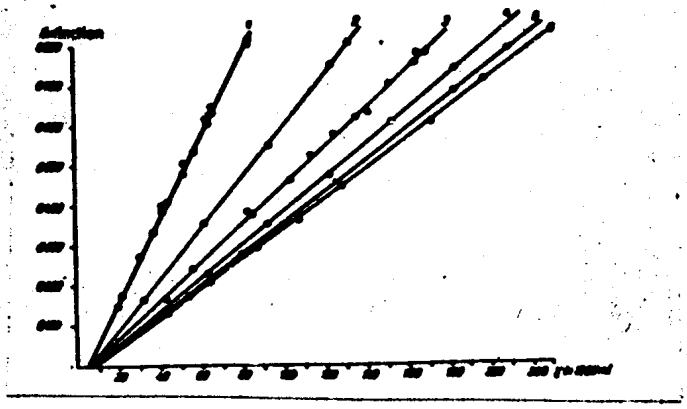

Figure 1

1, lysergic acid; 2, ergometrine maleate; 3, ergoclavine; 4, ergotinine; 5, Ergotoxine phosphate; 6, dihydroergotoxine phosphate

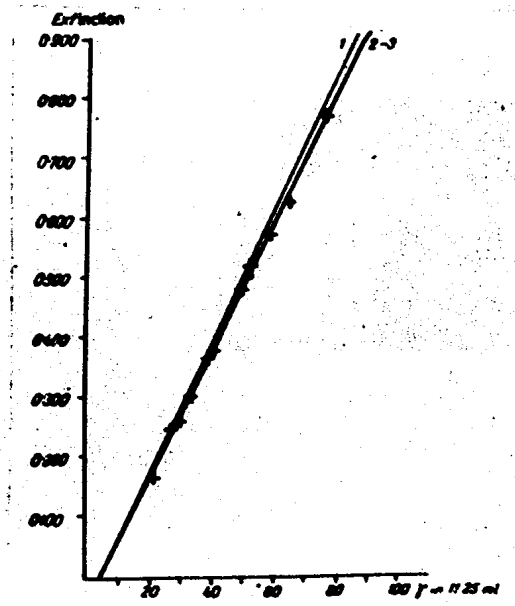

Figure 2

1, ergometrine maleate converted to lysergic acid; 2, lysergic acid; 3, + ergoclavine converted to lysergic acid

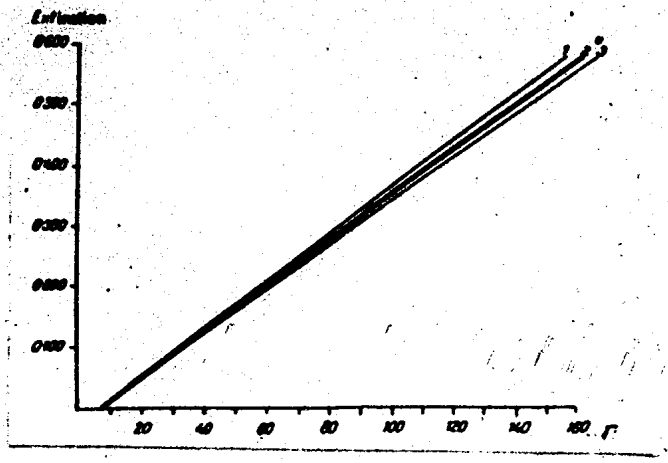

Figure 3

1, ergocornine phosphate, calculated; 2, ergotoxine phosphate from test results; 3, ergocristine phosphate, calculated

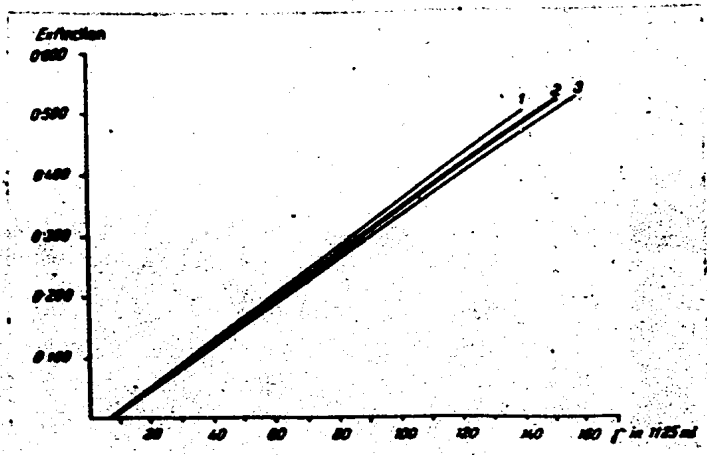

Figure 4

1, dihydroergocornine phosphate, calculated; 2, dihydroergotoxine phosphate, from test results; 3, dihydroergocristine phosphate, calculated